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# Closing the loop in bioproduction: Spent microbial biomass as a resource within circular bioeconomy

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ABSTRACT

Successful transition to a circular bioeconomy relies on the availability and efficient use of organic feedstocks such as agricultural and food waste. Advances in industrial biotechnology provide novel tools to valorize these feedstocks differently. Less attention, however, has been directed towards assessment of the organic side-residues arising from industrial biotechnology, such as spent microbial biomass (SMB). This study aims to reflect the current state of SMB within bioeconomy and create awareness of this growing industrial resource. Data from a range of published fermentation processes is used to estimate the amount of SMB formed per product (weight per weight, wt/wt) across different types of bioproducts, namely organic acids, alcohols, polymers, amino acids, antibiotics, protein and vitamins. Varying amounts of SMB are generated depending on the bioproducts and bioprocess, where bulk bioproducts, e.g. alcohols, generate less SMB than bioproduction of high-value low-volume specialty products, e.g. vitamins. It is estimated that more than 50 million tons of nutrient-rich SMB was generated in 2013, with SMB from bulk and specialty bioproduction accounting for roughly equal amounts. Furthermore, the composition of six industrially relevant organisms is summarized and compared, highlighting the general features of SMB as a carbon-rich substrate mainly consisting of protein. The results indicate that SMB is a growing resource with a reliable supply and predictable composition. The predictable nature of SMB could make it a favorable substrate for further innovation in industrial applications and nutrient circulation within the bioeconomy, for example, by using it as a co-substrate for valorization of other biomasses.

## Introduction

Global challenges such as climate change, loss of biodiversity and the growing human population are driving the transition from fossil-based economy to bioeconomy [1]. Bioeconomy utilizes renewable organic feedstocks to generate a spectrum of bio-based products by involving multidisciplinary areas of science and engineering [2].

Currently, bioeconomy within the EU generates about €2 trillion in annual turnover and is expected to grow, providing 19 million jobs by 2030 [2]. Successful transition to bio-based economy depends on feedstock availability, which must not compete with food production and preservation of natural ecosystems [3]. The key enabler for bioeconomy is industrial biotechnology (IB), which relies on capabilities of biological organisms to produce a range of useful products from different organic substrates (varying from agricultural products to food waste and beyond) [4]. IB can convert various feedstocks into biobased energy and biofuels, chemicals and bioplastics [5,6], pharmaceuticals [2], surfactants [7], food flavorings [8,9], pigments [10], biocellulose [11] and more. The diversity of IB processes and products is growing. Accordingly, the EU market for IB-derived products is forecast to reach €50 billion in 2030 [3]. Thus, IB can promote resource-efficient utilization of various renewable, organic feedstocks and advance biomass-efficient circular bioeconomy. For a complete nutrient circulation within circular bioeconomy, the nutrients and organic side-streams from IB bioproduction processes (e.g., microbial biomass, wastewaters) should also be reused.

Microbial biomass is an integral part of any microbial bioproduction process, where live microorganisms are used to transform organic substrates into useful products. For this study, any such stage within bioproduction process will be referred to as “fermentation”. In some cases, the microbial biomass itself can be the product (e.g., probiotic bacteria, dried yeast for baking or brewing). However, most industrial

*Abbreviations:* SMB, spent microbial biomass; wt/wt, weight per weight; IB, industrial biotechnology; DCW, dry cell weight; Mt, million tons; CAGR, compound annual growth rate; TRL, technology readiness level; PHB, polyhydroxybutyrate; GMO, genetically modified organism; SCP, single cell protein.

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bioproducts are compounds secreted outside cells and then purified from cultivation media. Thus, product recovery often starts with cell separation from the liquid broth, and the remaining cells (i.e., spent microbial biomass, SMB) are a common bulk fermentation residue [4].

With the global transition from fossil economy to bioeconomy, increases in the diversity and volume of chemicals and products derived using cell metabolism will take place [12]. As the consequence, generation of industrial SMB will also increase. Currently, most research attention has been diverted towards valorization of one type of SMB - spent brewer's yeast. As the result, several valuable reviews are available on the amount and valorization of yeast [13–16]. However, information on other types of SMB and their production volumes is sparse.

This study aims to reflect the current state of SMB within the bioeconomy and to create awareness of this growing industrial resource. First, an estimate of the amount of SMB production for different types of bioproducts is presented, by analyzing the data in academic publications to estimate the amount of SMB (dry cell weight, DCW) formed per unit of fermentation product (wt/wt). This approach allows extraction and comparison of publicly available data from a range of fermentation processes and bioproducts reported in academic studies. The caveat is that the process parameters from the majority of these studies are different from industrial operations. Nevertheless, the obtained estimates provide an insight into SMB formation across different types of bioproducts in the absence of surveying industrial practice across the bioindustries, beyond the scope of this study. To the best of our knowledge, this is the first such attempt, and is accompanied with a discussion on the challenges and considerations associated with such a task. The composition of a subset of common SMB microorganisms is then reviewed, followed by a summary of existing and potential SMB valorization and considerations to provide a context for the part SMB could play within the growing circular bioeconomy.

## Methods

### *Estimating amounts of SMB*

The amount of SMB formed depends on the bioproduct and the fermentation process. In this study, the term 'fermentation process' includes the diversity of biological and process-environment factors (e.g., aerobic or anaerobic conditions, fermentation substrate, actively growing or stationary cells [4,17,18]) that influence the yield of the bioproduct and the yield of SMB. Product yield (i.e., amount of product per unit of substrate) is often used to compare different processes for the same product. A similar concept of biomass yield can be introduced to compare cell growth on various substrates. It must be noted, that strong coupling exists between fermentation substrate, product yield and SMB yield. In short, substrate is required for formation of both the products and biomass, whereas biomass acts as a driver to product formation. As the aim of this study was to estimate SMB formation across a variety of bioproducts and substrates, SMB formation was assessed relative to the product formation.

To understand SMB formation across the breadth of the bioeconomy, reports of fermentation processes were searched using academic data bases such as Scopus and Web of Science. Given the ample range of products and active innovation in the fields of bioproduction, the scope of this work is framed around a selected subset of bioproducts from different market categories, namely alcohols, amino acids, organic acids, polymers, vitamins, antibiotics, and protein.

The caveats of this approach originate from the fact that academic work generally concerns the early stages of technological development (TRL 3–5), and the process conditions are far from the largescale operations required for industry. This encumbers data extraction, because the early TRL studies focus on a range of different process parameters (microbial strains, feedstocks, temperature, pH, reactor type etc.)[19, 20] to increase and optimize product yield. The formation of by-products such as SMB is secondary, hence the amount of cell growth is often either omitted or reported using study-specific parameters such as optical density. In addition, the various process parameters being explored can influence the microbial metabolism creating a range of different SMB-to-product ratios. To gain a more comprehensive assessment of SMB to product formation, bioproduction processes from different published sources were analyzed for each selected bioproduct. More details on the considerations for data sources and consultation process are described in Supplementary Information.

The SMB was defined as the amount of microbial biomass as dry cell weight (DCW) at the end of the process. For polyhydroxybutyrate (PHB), which is an intracellular product, the SMB was calculated as biomass remaining after product removal, i.e. 'mass of DCW' – 'mass of PHB'. Firstly, the SMB to product ratio was calculated for each process. Due to differences in process conditions, such as host microorganism, substrate, temperature, pH etc., the ratios showed considerable variability. The representative ratio for each product was calculated as a median value from all production processes analyzed for that product. This was done to limit the influence of processes with extreme cases of SMB to product ratios, with the assumption that the ratio for industrial processes is likely to verge in the lower or middle range because of their optimization and intensification. To inform on SMB formation across the breadth of the bioeconomy, the selected bioproducts were grouped into key market categories, namely alcohols, amino acids, organic acids, polymers, vitamins, antibiotics and proteins. An average of the grouped SMB to product ratio was then calculated for each one. A graphic illustrating SMB to product estimation process is shown in a Supplementary Fig. S1.

Due to the limited availability of comprehensive data on cell growth over the course of the fermentation, this analysis primarily focuses on batch and, where possible, fed batch processes without considering SMB reuse or recirculation, which can be significant. In addition, it has not included gas fermentation processes, where acetate and ethanol are produced from gases such as CO, $CO_2$ and $H_2$ [21]. These processes use very slow growing bacteria such as *Acetobacterium woodii* and *Clostridium autoethanogenum*, where almost all microbial growth is used to regenerate itself [21].

### *Considerations for SMB composition estimates*

The microorganisms forming the SMB can vary depending on bioproduct and production process (Supplementary Table 1). To consider SMB composition across various bulk and specialty products, a set of representative SMB organisms were selected to estimate their biomass composition. They are *E. coli*, *Corynebacterium glutamicum*, *Streptomyces sp.*, *Aspergillus niger* and *A. terreus*, *Saccharomyces cerevisiae* and *Pichia pastoris*. Further information on which organisms represent which bioproduct group, as well as an illustrative estimate of their annual production volume is shown in Supplementary Table 2.

The SMB composition of a microorganism can vary with the type of substrate, the specific microbial strain and culture conditions, e.g., the protein content of *A. niger* can vary from 7 % to 42 % [22]. To generate an estimate of average SMB composition, an average mass (g/100 g DCW) of each macromolecular component was calculated using data from several studies. Biomass composition is reported for the main categories of macromolecules - protein, lipid, DNA, RNA, carbohydrates, peptidoglycan, and ash. In some cases, where other components such as small molecules (cofactors, polyamines) were reported, these are included in the category "Other". Wherever possible, data from analytical biomass studies were used. In other cases, biomass composition was extracted from published genome scale metabolic models of the organism, as these are often built integrating a wide range of available information and their predictive accuracy tested experimentally.

## Results and discussion

### *SMB production volumes reflect the diversity of bioproducts*

This section demonstrates the high variation in SMB amount resulting from production of diverse bioproducts. The selected bioproducts and results of SMB to product ratio calculations are shown in Supplementary Table 1. Fig. 1 compares SMB formation across various product groups. The black lines represent the calculated average SMB to product ratio as presented in Supplementary Table 1. The scattered dots give an indication of heterogeneity in SMB formation across different bioprocesses and products within each group, which may span more than two orders of magnitude. Each product within the group has its own color. For example, amino acids are represented by four amino acids, where glutamate production (green dots) generate less SMB than production of methionine (yellow dots) spanning the SMB to product ratio range of 0.9–7.4. Similar diversity in SMB production between different products is seen within other product groups. Thus, production of vitamins B12 and K2 can produce 10-times more SMB than some of the processes for B2 production. Propanediol generates more SMB than most ethanol and beer fermentation processes. The least amount of SMB is accumulated in acetate production. In addition, production of PHB and ethanol can generate very different amounts of SMB depending on the production process and fermentation feedstock, e.g. some feedstocks require fewer cell resources to support growth and to be transformed into the product more easily, thus more product is obtained per SMB. In this analysis, "Polymers" are represented by only one compound, PHB, a biodegradable polyester polymer which is seen as a sustainable replacement candidate for fossil commodity polymers such as polypropylene [23]. PHB bioproduction uses microorganisms that synthesize and accumulate it within their biomass. Other notable biopolymers are polylactic acid and polyethylene, which are derived from lactic acid and ethanol [6]; both are included in this analysis as organic acids and alcohols, respectively.

It can be observed that production of specialty bioproducts, such as vitamins, protein, and antibiotics, generates higher amounts of SMB per unit weight of product than bulk chemicals such as alcohols, organic acids and amino acids. Thus, the highest SMB production amounts were estimated to be 110 kg per kg of vitamins, while the lowest were 0.1 kg per kg of organic acids. This illustrates the difference in biological production mechanisms. In general, specialty products require more cellular resources (more enzymes, longer metabolic pathways) than production of bulk products, which are generally derived from central cell metabolism. In fact, most bulk products generate less biomass than product, while amino acid production generates roughly equal amount of biomass and product, and specialty products can generate 10–100 times more SMB than product. The trends in Fig. 1 are observed in some of the industrial reporting, e.g. comparing production of organic acids, alcohols and amino acids, where succinate production generates about 0.10 wt/wt SMB [26], propanediol production generates about 0.27 wt/wt SMB [27], while production of glutamate-based umami generates ≈ 3.2 wt/wt organic co-product [28] likely to consist of SMB along with other processing side-residues. It can be noted that in these industrial cases the SMB generated per unit of product is within the same order of magnitude as estimates from this study. However, caution has to be exercised when comparing estimates from this study to industrial processes, as little is known of how SMB to product ratios change over the course of process scale-up. On the one hand, the industrial processes undergo process optimization and intensification to increase product yield, while on the other it is a 'harsher' microenvironment, e.g. due to

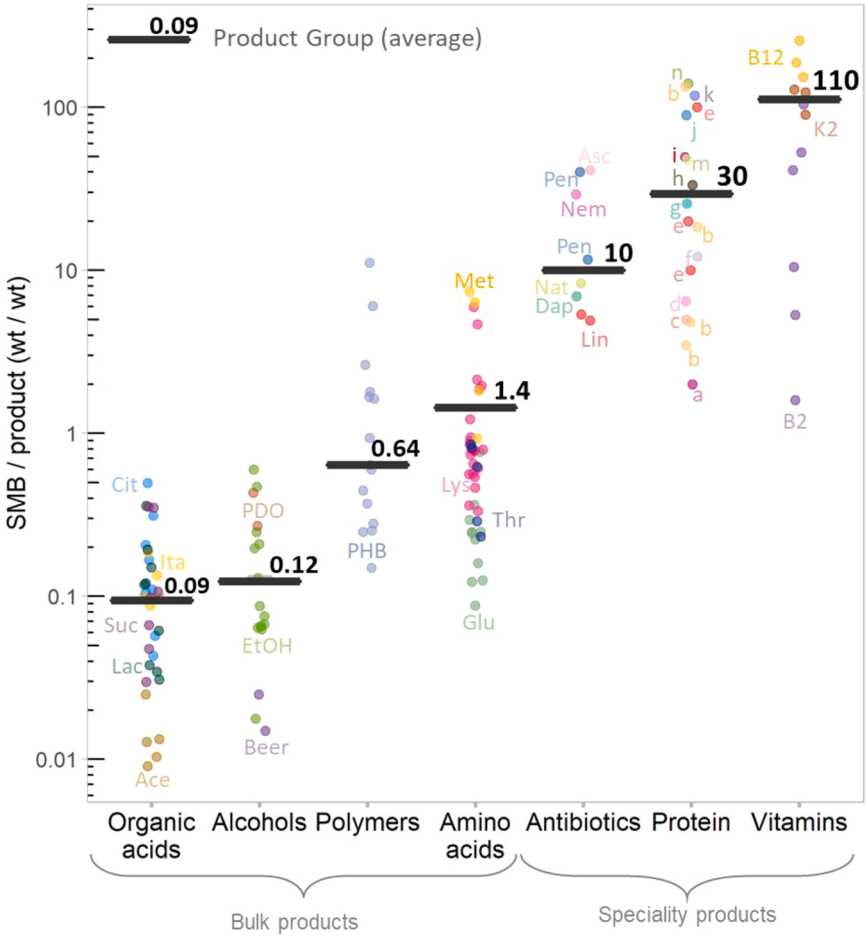


**Fig. 1.** SMB formation in various groups of bioproducts represented as SMB to product weight ratio. Due to the high diversity of the quantity of SMB formed per unit of product, a log transformation was used for the y-axis. The black lines represent the average value of products within the product group as shown in Supplementary Table S1. As polymers are represented by only one bioproduct - polyhydroxybutyrate (PHB) – the black line represents the median value of all processes within dataset. The scattered dots indicate datapoints from individual production processes. Datapoints for the same product within a product group are shown in the same colour (e.g., acetate, Ace, within organic acids, tan dots). Other organic acids are: Cit - citrate, Ita - Itaconite, Lac - Lactate, Suc - Succinate. Alcohols: EtOH - Ethanol, PDB - 1,3-Propanediol. Amino acids: Glu - Glutamate, Lys - Lysine, Met - Methionine, Thr - Threonine. Antibiotics: Asc - Ascomycin, Dap - Daptomycin, Lin - Lincomycin, Pen - Penicillin, Nat - Natamycin, Nem - Nemadectin. Protein products included are: a - Cyclodextrin glycosyltransferase; b - Insulin; c - Cellulase; d - Pneumococcal surface protein A vaccine; e - Glucosidase; f - Interferon; g - Interleukin 1; h - Growth factor; i - Interleukin 2; j - Amylase; k - Parathyroid hormone; m - Mini antibody; n - Lipase (see details in Supplementary Table 1). For simplified data visualization, two points representing SMB to product formation for trypsin production are excluded. These are 730 wt/wt from [24] and 1600 wt/wt as reviewed in [25]. Vitamins: B2 - Riboflavin, K2 - Menaquinone, B12 - Cobalamin.

changing substrate concentrations due to uneven mixing [29], for cells to grow than in the laboratory-scale studies.

### *SMB is significant and growing industrial residue*

The SMB to product ratio obtained from Fig. 1 was then applied to production volumes, with cross-sector data publicly available for year 2013 [6], to estimate the quantity of SMB generated (Fig. 2; note the square-root transformation on the y axis). These numbers are intended to give an impression of the order of magnitude in which various bioproducts are on the market and what the amounts of their corresponding SMB. One should be aware that reliable market and industrial data are rarely found in the public domain. As seen with SMB to product ratios, overall specialty products generate more SMB than product, whereas bulk products create less residual SMB than product. Thus, the highest volume of annual SMB production, 20 Mt, is estimated for vitamin production, despite having one of the smallest production volumes, 0.2 Mt, reflecting the high SMB to product ratio observed earlier. Vitamin production is estimated to generate twice as much SMB as alcohol production, which has the highest production volume of 100 Mt. These estimates indicate that production of bulk commodity products and specialty products generate roughly equivalent volumes of SMB, i.e., 22 and 27 Mt, respectively. The total estimated annual SMB volume for the product groups considered is 50 Mt (2013 figure).

This appears to be the first attempt to estimate how much SMB is generated within the growing biotechnology. The estimated amount of 50 Mt might seem small in comparison to many other organic waste streams such as food waste, estimated as 1300 Mt/a globally [30], or lignocellulosic waste, where corn stover alone account for 1300 M t/a [31]. However, considering global transition from fossil-based to bio-based production, the volume of bioproduction and SMB generation is likely to increase [32]. A significant market growth is predicted for both bulk and specialty bioproducts. The global bio-based chemicals market is expected to increase with a compound annual growth rate (CAGR) of over 10.0 % from 2019 to 2025 [33], whereas CAGR for such specialty products as vitamins and antibiotics is set at 6.13 % [34] and 4.5 % [35] from 2021 to 2028, respectively. The CAGR for biopharmaceuticals is estimated as 13.8 % from 2018 to 2025 [36]. It is also expected that the growth in bioeconomy will be supported by development of new bio-valorization pathways for the various biowaste streams, such as the aforementioned food waste and lignocellulosic waste [37], hence increasing the amount of fermentation products, thus also SMB. Considering these projections, SMB can be regarded as an organic industrial residue with a considerable volume.

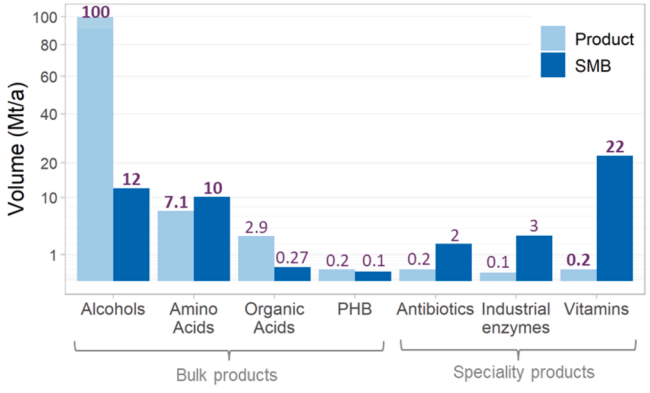


**Fig. 2.** Annual production of selected bioproduct groups and the estimated volume of SMB generation in million metric tons per year (Mt/a). Light Blue - product groups, Dark blue - estimated SMB amount. Production volume data reported in [6] are used together with SMB to product average ratio shown in Fig. 1 and the supplementary Table S1. Due to the high range of product and SMB volumes, a square root transformation was used for the y-axis. Polymers are represented by only one bioproduct - polyhydroxybutyrate (PHB).

In addition, due to the industrial nature of SMB generation, it is a stable and predictable resource. Industrial fermentation is a highly regulated and monitored process, where measurement of such process parameters as temperature, pH and substrate supply help to ensure predictable fermentation outcomes [4]. For the same reasons, one can expect SMB composition and supply to be reliable. For example, around 48 Mt of SMB are produced daily throughout the year in a 1,3-propanediol fermentation plant [27]. Such steadiness differs from most other types of biomasses. Lignocellulose or food waste can be very heterogenous feedstocks and their availability can vary significantly across seasons [38,39].

### *SMB composition estimates*

The SMB valorization options depend on the SMB properties and composition. Fig. 3a shows an estimate of macromolecular composition for selected common SMB organisms. In general, all microorganisms have a similar macromolecular composition, with the main biomass fraction being composed of proteins (40–60 %). This is consistent with estimates of the protein content as 50–80 % for bacteria and 30–70 % for fungi [40]. Remaining biomass is composed of polysaccharides (1–30 %) and lipids (1–15 %), RNA (4–17 %) and ash (6–15 %). All selected bacteria (*Streptomyces sp.*, *E. coli* and *C. glutamicum*) have higher RNA (9–18 %) and DNA content (2–4 %), while yeasts and the *Aspergillus sp.* have only 5–6 % and 0.3–1 %, respectively. The other differences in their biomass reflect the diversity in the composition of microbial cell walls. Thus, fungal cell walls are composed primarily of polysaccharides, such as $\alpha$-glucans, $\beta$-glucans, and chitin [16,41,42], whereas bacterial cell walls contain a peptidoglycan layer, which is thicker for Gram-positive bacteria (*S. coelicolor* and *C. glutamicum*) than Gram-negatives (*E. coli*) [43]. In addition, *E. coli* and *C. glutamicum* biomass has a higher lipid fraction than others. The amount of ash is similar among all organisms (6–10 %).

The information of average macromolecular composition in Fig. 3a can be used to estimate elemental composition. This was done using average macromolecular stoichiometries as follows: $C_5H_7O_2N$ for protein, $C_6H_{10}O_5$ for carbohydrates and $C_{57}H_{104}O_6$ for lipids [65], $C_{19.5}N_{7.5}P_{5.2}$ for DNA and RNA [66], and for peptidoglycan $C_{37}H_{71}N_7O_{26}$ (*E. coli*) [67], $C_{40}H_{78}N_8O_{28}$ (*C. glutamicum*) [52] and $C_{148}H_{234}N_{28}O_{79}$ (*Streptomyces sp.*) [56]. The calculated elemental composition of SMB is shown in Fig. 3b. As these estimates are approximated from the available data on main macromolecular components, their accuracy is limited. For example, this approach seems to overestimate the amount of phosphorus (P) as seen when comparing the estimated composition of *S. cerevisae* with previous reports [68,69]. Although these studies report similar biomass composition overall, reported biomass contains less phosphorus (1.0 instead of 3.3 g/100 g DCW) and more oxygen (36 instead of 24 g/100 g DCW). Similar discrepancies were observed for *C. glutamicum* composition, where previous studies [51,54] indicated more oxygen (28 g/100 g DCW), hydrogen (6.6 g/100 g) and nitrogen (13 g/100 g), and less phosphorus (2.6 g/100 g) than estimated from macromolecular composition (23, 5.6, 10 and 4.9 g/100 g DCW, respectively). Despite these limitations, Fig. 3b shows that all selected types of SMB are composed mostly of carbon and oxygen accounting for 40–55 g/100 g DCW and 20–32 g/100 g DCW, respectively. Nitrogen accounts for about 6–13 g/100 g DCW and the average C/N ratio ranges from 3.8 to 7.8. In addition, bacteria have more nitrogen and phosphorus compared to fungi, which reflects their higher RNA and DNA content.

### *Summary of potential SMB valorization routes*

Fig. 3 indicates that SMB is a nutrient and carbon-rich substrate. Thus, SMB management plays an important part in the industrial process design, as SMB must be treated appropriately to limit any potential

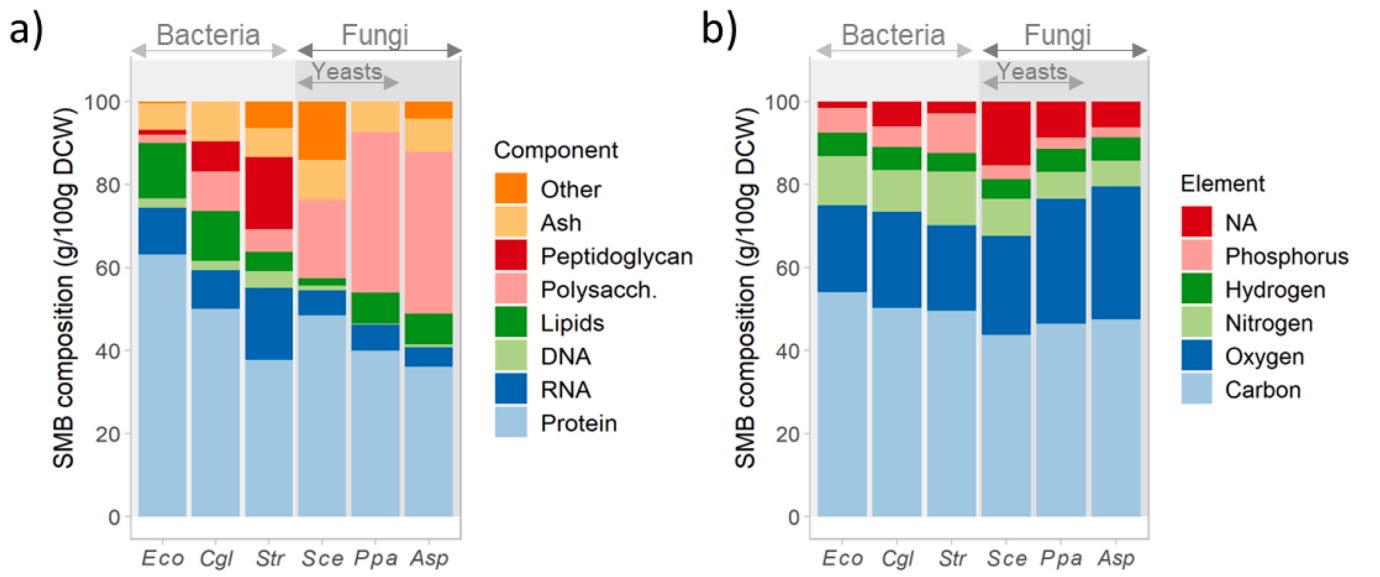


**Fig. 3.** Estimated macromolecular (a) and elemental (b) composition of SMB from selected microorganisms. DCW - Dry Cell Weight. (a) Data represent calculated average for each biomass component based on published literature on *S. cerevisiae (Sce)* [13,44–46], *E. coli (Eco)* [47–50], *C. glutamicum (Cgl)* [51–54], *Streptomyces sp. (Str)* [50,55,56], *P. pastoris (Ppa)* [57–61] and *Aspergillus sp. (Asp)* [62–64]. (b) The elemental composition of SMB was derived from macromolecular composition reported in (a) using an estimated average stoichiometry for macromolecules: $C_5H_7NO_2$ for protein; $C_6H_{10}O_5$ for carbohydrates; $C_{57}H_{104}O_6$ for lipids [65], $C_{19.5}N_{7.5}P_{5.2}$ for DNA and RNA [66], and for peptidoglycan - $C_{37}H_{71}N_7O_{26}$ (*E. coli*) [67], $C_{40}H_{78}N_8O_{28}$ (*C. glutamicum*) [52] and $C_{148}H_{234}N_{28}O_{79}$ (*Streptomyces sp.*) [56]. Category "NA" is used to visualize the SMB fraction formed by molecules not accounted within the main groups of macromolecules.

adverse environmental effects. In addition, nutrient recycling through various SMB valorization routes can help to reduce the organic content of SMB and generate added-value products. Here a brief outlook is provided on some of the SMB valorization options and potential opportunities for innovations, while a detailed discussion is outside the scope of this work.

Some types of SMB, e.g. brewers yeast, are already recycled in a range of established valorization routes. These include SMB use as an agricultural fertilizer [28,49,70], biogas[71,72], food and feed [13]. In addition, yeast extract is a common ingredient in growth media for various fermentation processes, where it can act as both carbon and nitrogen source [73]. Among different SMBs, spent yeast has been investigated the most for various exploratory valorization routes such as a source of enzymes and nucleic acids, as a substrate to grow edible insects, for biosorption of heavy metals, as foaming agent for concrete and others (see review in [13]). Considering the relative similarity of yeast biomass with other SMB organisms, it would be useful to investigate if these valorization options could be appropriate for other types of SMB as well.

Another route for SMB valorization is co-production of the so-called 'single cell proteins' (SCP), i.e. proteins produced in various algal, bacterial and fungal cells, alongside other microbial products (enzymes, organic acids, antibiotics etc.)[74]. The use of SCPs as animal and aquaculture feed is growing (see [74] for a review on various organisms, types of SCP and industrial production), and exploration of SCP production from SMB as a valorization opportunity is likely to follow. It should be noted that not all SMB would be suitable for feed applications due to unsuitable nutritional quality, including poor concentration of macro-and micronutrients, or presence of anti-nutritional compounds such as bacterial toxins or mycotoxins [39,74]. One option for such SMB could be to test it as a substrate for growing other edible organisms, such as spirulina and yeast, or insects [39,74].

Given the high protein content of SMB, it can be utilized alongside other protein-rich substrates within the developing area of protein-biorefinery, where amino acids and oligopeptides are extracted from protein-rich substrates for the production of bio-based chemicals, such as polymers, commodity chemicals, pharmaceuticals and other fine chemicals [75]. (For a comprehensive review on process steps required to use protein-rich biomass wastes as potential feedstocks for the chemical industry see [75]). Another exploratory idea is to use fermentation to convert amino acids to higher alcohols. In this case genetic engineering is used to generate microbial strains able to metabolize and deaminate amino acids for co-production of ammonia and biofuel [73,76–78]. For example, engineered *E. coli* has been used to bioconvert the biomass of *S. cerevisiae*, *E. coli*, *B. subtilis* and microalgae to produce alcohols [76], and a more recent review can be found in [78].

It should also be noted that genetically modified organisms (GMO) play a significant part in industrial biotechnology. Public opinion on the acceptable use of GMO varies across the world [74]. GMO regulations commonly also specify the treatment of GMO wastes, and they commonly undergo cell inactivation by heat, chemical, treatment, or physical disruption [70,79]. It can be envisioned that valorization of SMB originating from GMO will require additional evaluation on a case-by-case basis. This will assess the safety and appropriateness for the intended valorization, such as safety and nutritional quality for feed or food applications, and possible amendments of treatment, including additional steps to ensure removal of recombinant DNA [80]. Cell inactivation resulting in cell lysis can also be beneficial for several valorization routes, as the cell contents are released, and the cytoplasmic nutrients are more accessible to plants and other organisms, if used as fertilizer or as substrate for feed or fermentation.

Further research on SMB suitability for a particular valorization route would help to guide resource and nutrient circulation within the bioeconomy. The predictable nature of SMB could make it a favorable substrate for further innovations in industrial SMB applications and contribute to industrial symbiosis. In fact, it could even be suggested that potential SMB valorization could be considered already during the early stages of bioprocess and strain development. This would allow use of the tools of synthetic biology and metabolic engineering to optimize not only the fermentation yield of the intended product, but also to add value to the contents of the unavoidable SMB by-product making it a more compatible and attractive substrate for subsequent valorization cascades. For example, the strain could be modified to improve its nutritional quality or amino acid content alongside production of the product compound [74]. This is on a par with the proposed concept of Sustainable Metabolic Engineering [12], where an assessment and

optimization of economic, environmental, and societal sustainability parameters are taken into account during the early stages of metabolic engineering and biotechnology strain development.

## Conclusion

This study analyzes a number of published fermentation processes for production of bioproducts ranging from organic acids and alcohols to industrial enzymes and vitamins with the aim of estimating the amount of a key industrial side-product – spent microbial biomass (SMB) consisting of microbial cells generated during fermentation. This resource is dominated by microorganisms used widely for bulk production (e.g., yeast for bioethanol, *Clostridium glutamicum* for amino acids) or specialty products, where more biomass is generated per unit of product, as in, vitamin and enzyme production. Irrespective of the type of industrial microorganism, SMB is rich in protein and cell wall materials. Due to the nature of industrial SMB, it is produced in stable amounts with a predictable SMB content. Currently, research on SMB valorization focuses on one type – brewer's yeast. Given the increasing use of industrial biotechnology and SMB as an increasingly available resource, other types of SMB should also be explored for their suitability to different valorization routes, ranging from agricultural fertilizer and animal feed to higher value-added product extraction (biorefinery).

To promote concerted research and development efforts for SMB valorization, the research community would benefit from better mapping and awareness of industrial SMB types, as well as sharing experiences of current SMB treatment and valorization strategies. This would create fertile ground to further research efforts for understanding what makes each type of SMB suitable for different valorization routes, as well as addressing technical capabilities and practical issues of SMB handling. In addition, mapping of available industrial side-streams would help to comprehend the resource potential of other types of industrial cell biomasses such as mammalian cell lines used in biopharmaceutical production and microalgae used for biofuel, pigments and others.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

## Acknowledgements

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.nbt.2022.06.001.

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